Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60270-4

Antihyperglycemic and *in vivo* antioxidative activity evaluation of *Cinnamomum bejolghota* (Buch.-Ham.) in streptozotocin induced diabetic rats: an ethnomedicinal plant in Assam

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ARTICLE INFO

Article history:
Received 22 Jul 2014
Received in revised form 18 Aug 2014
Accepted 17 Sep 2014
Available online 29 Sep 2014

Keywords:
Cinnamomum bejolghota
Anti-hyperglycemic
Antioxidant
Histopathology

ABSTRACT

Objective: To evaluate the antihyperglycemic property of *Cinnamomum bejolghota* (Buch.-Ham.) on streptozotocin induced type-2 diabetic rats.
Methods: Oral glucose tolerance test level was measured at 0, 30, 60, 90 and 120 min after the administration of extract. The extract was orally administered once daily at two dose levels of 250 and 500 mg/kg for 15 d. The effect of methanolic extract of *Cinnamomum bejolghota* (MECB) on the divergence of body weights, blood glucose levels and the biochemical parameters *viz.*, total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, aspartate transaminase, alanine transaminase, alkaline phosphatase were measured in an autoanalyzer. Histopathology of pancreas and *in vivo* antioxidative status was studied.
Results: A significant increase in bodyweights and rapid decrease in hyperglycemic peak was experiential in animals treated with MECB. After 15 d treatment the total cholesterol, TG, low density lipoprotein level decreased and high density cholesterol level increased significantly. MECB reduced the levels of the elevated marker enzymes aspartate transaminase, alanine transaminase and alkaline phosphatase. MECB reduced the lipid peroxidation and improved the level of catalase and glutathione in liver. Histopathological studies of pancreas in diabetic and treated groups substantiate the cytoprotective action of extract.
Conclusions: It can be evident from the research work that *Cinnamomum bejolghota* (Buch.-Ham.) has potent antihyperglycemic activity and supports the *in vivo* antioxidative status.

1. Introduction

Diabetes mellitus is the most significant chronic disease and a growing health problem in most countries[1-3]. It is due to lack of insulin secretion in β- cells of pancreas and desensitization of insulin receptors for insulin[4]. It causes a number of complications such as retinopathy, neuropathy, and peripheral vascular insufficiencies[2]. Most patients with type-2 diabetes have insulin resistance and this is predisposing to both diabetes and cardiovascular disease[3] which usually results from high level of glucose in the blood and byproducts of lipid metabolism within the tissues[4]. Many oral hypoglycemic agents, such as biguanides and sulfonylurea are available along with insulin for the treatment of diabetes mellitus but these synthetic agents can produce serious side-effects, and in addition, they are not suitable for use during pregnancy[5,6]. Recently, the search for appropriate antihyperglycemic agents has been focused on the types of natural food products used in traditional medicine. Many traditional medicinal herb extracts have been used for the treatment of diabetes mellitus due to lower side effects. Many researchers have shown that cinnamon extract has a moderate effect in reducing fasting plasma glucose concentrations in both diabetic patients and animals[7-10]. The active compounds of cinnamon have been reported, such as water-soluble polyphenol type-A polymers[11,12], cinnamaldehyde[13], and cinnamic acid[14].

Cinnamomum bejolghota (Buch.-Ham.) (*C. bejolghota*) is a

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Foundation Project: Supported by Research Promotion Scheme 2011, Grant No.: 8023/RIB/RPS-10/(NER)/2011-12.

medium- to large-sized evergreen tree with aromatic leaves, stem bark and panicle, and distributed in the central and outer parts of eastern Himalayas up to an altitude of 2 100 m, and also in Andaman Islands. In Assam the plant is well distributed in the Jorhat, Sibsagar, Golaghat, Nowgaon and Kamrup districts. It also grows in the Khasi, Garo and Jaintia districts of Meghalaya and in a few places of Nagaland in northeast India[15]. The bark, which is sold at the local markets, is used traditionally in the region as a spice. The bark and its infusions have local medicinal use for the treatment of a cough, cold, toothache, liver complaints, diabetes, gall stones and are used as a mouth freshener. In contrast, the leaves have been in use among some of the ethnic societies for preparation of a special kind of rice-beer known as "Apong"[16]. Work has been carried out with the methanolic bark extract of *C. bejolghota* (MECB) with a view to investigate its anti-diabetic potential in streptozotocin (STZ) induced type-2 diabetic rats against metformin as a standard drug.

2. Materials and methods

2.1. Chemicals and diagnostic kits

STZ, ethyl ether, ethylenediaminetetraacetic acid disodium salt, *n*-butyl alcohol, pyridine, reduced glutathione (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), carboxy methyl cellulose, acetic acid, tris buffer (Rankem Chemicals, Faridabad, India), metformin (Sigma- Aldrich Chemical Company, St. Louis, M.O, USA), potassium dihydrogen phosphate, disodium hydrogen phosphate, thiobarbituric acid (HiMedia Labotratory, Mumbai, India), hydrogen peroxide (Loba Chemie Pvt. Ltd., Mumbai, India), 5,5-dithiobis (2-nitrobenzoic acid), sodium dodecyl sulphate (Otto Chemie Pvt. Ltd., Mumbai, India), trichloroacetic acid (Qualikems fine chemicals P. Ltd., New Delhi, India), potassium chloride (Spectrochem Pvt. Ltd., Mumbai, India), one-touch glucometer accucheck (Roche products, Pvt. Ltd., Bayer Diagnostics, Mumbai, India), alkaline phosphatase (ALP) kit, total cholesterol kit, high density cholesterol (HDL) kit, triglyceride kit, alanine transaminase (SGPT) Kit, aspartate transaminase (SGOT) kit (Span Diagnostics Pvt. Ltd., Surat, India) were used in this study.

2.2. Collection of plant material and preparation of methanolic extract obtained from the plant bark

The bark of *C. bejolghota* (Buch.-Ham.) was collected from Rowriah, Jorhat, Assam, India during the month of July, 2013. The plant was identified and authenticated by Dr. A.A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (specimen No. DU/CB/2014/07, reference No. (BSI/ERC/2014/Plant identification/882) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. Barks were cut into pieces, washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried bark pieces were then pulverized in a mechanical grinder to coarse powder and then stored in airtight containers free from moisture.

Powdered crude drug (250 g) of *C. bejolghota* bark were extracted by Soxhlation (Continuous hot extraction) with 1 000 mL of methanol for 18 h after pretreatment with petroleum ether. The solvent was recovered at 50 °C by distillation under reduced pressure and the extract was concentrated to obtain an orange brown semisolid mass. Preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents. The methanolic extract of the bark of *C. bejolghota* (MECB) contains flavanoids, carbohydrates, glycosides, lignin, steroids, saponins, tannins and phenolic compounds.

2.3. Drugs

Methanolic bark extract of *C. bejolghota* (250 and 500 mg/kg of body weight), metformin (10 mg/kg of body weight per orally) were used in the experiment.

2.4. Selection and maintenance of animals

Male albino rats of Wistar strain, weighing about 100-200 g were obtained from M/S Chakraborty Enterprise, Kolkata and used for the experimental study. The required approval for performing the animal study was acknowledged from the Institutional Animal Ethical committee, Dibrugarh University, vide registration number-1576/GO/a/11/CPCSEA dated 17/2/2012, and approval number-IAEC/DU/59 dated: 24/9/2013.

The animal house was well ventilated and maintained at room temperature from (20±2) °C to (25±2) °C, 30%-35% of relative humidity and 12 h dark/light cycle. They were housed in large spacious hygienic cages during the course of the experimental period and were provided with pellet diet (Hindustan lever, Mumbai, India) and water *ad libitum*. Prior to the experimental study, animals were fasted by depriving them of food for 16 h but allowing free access to water. The place of experiment was kept very hygienic by cleansing with antiseptic solution, and further procedures involving care of animals was conducted in conformity with the institutional guidelines.

2.5. Acute toxicity study

Healthy adult male Wistar rats were starved overnight and divided into five groups containing five animals in each group (n=5). They were orally fed with MECB dissolved in 0.5% carboxy methylcellulose in increasing dose levels of 500, 1 000, 1 500, 2 000, 2 500 mg/kg. The animals were observed continuously for 2 h and then for 4 h under behavioral (gross behavior, writhing, convulsion, response to tail pinching, pupil size, itching, excessive salivation, urination, faecal output, water intake, feeding behavior, sedation *etc*.), neurological and autonomic profiles. After a period of 24 and 72 h, they were observed for any lethality or death and further kept under observation up to 15 d. The effective dose of the MECB was determined to be $1/10^{th}$ of the maximum dose which is 2 500 mg/kg[17].

2.6. Oral glucose tolerance test (OGTT)

Initial screening of the extract for the hypoglycemic activity was done in normal healthy rats by conducting OGTT. The OGTT was performed for two different doses of MECB (250 and 500 mg/kg of bodyweight per orally) and blood glucose level was measured by one touch glucometer (accu-check). The glucose level was measured at the intervals of 0, 30, 60, 90 and 120 min after the administration of extract[18].

2.7. Induction of experimental diabetes

After overnight fasting, type 2 diabetes was induced by intraperitoneal injection of STZ dissolved in 0.1 mol/L cold citrate buffer, pH 4.5, at a dose of 55 mg/kg of body weight. The control rats received the vehicle alone. After 1 week diabetes was developed. The rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

2.8. Experimental design

The experimental rats were divided into five groups with 5 rats for each. Group-I was administered with 0.5% carboxy methylcellulose 5 mL/kg body weight orally as normal control. Group-II was received 55 mg/kg body weight STZ with diabetic control rats. Group-III was treated orally once a day for 15 d with MECB, 250 mg/kg body weight and served as low dose. Group-IV was treated orally once a day for 15 d with MECB, 250 mg/kg body weight and served as high dose. Group-V was treated orally once a day for 15 d with metformin 10 mg/kg of body weight and served as standard control.

Blood samples were collected from the tail vein of the overnight (12-15 h) fasted rats and blood glucose level was determined on 0th, 5th,10th and 15th d. On the 15th day all the animals were sacrificed and evaluated for the biochemical parameters, histopathology and *in vivo* antioxidative status[19].

2.9. Biochemical estimations

After 15 d of treatment, the animals were fasted for 15 h and then sacrificed by cervical decapitation. Blood was collected in the tubes containing ethylene diamine tetraacetic acid as anticoagulant for the estimations of total cholesterol, HDL, low density lipoprotein (LDL), triglycerides, in plasma and SGOT, SGPT, ALP levels were estimated in serum that was collected separately. These parameters were measured in a colorimeter.

2.10. In vivo antioxidative status

A portion of the liver tissue was dissected out, washed with ice cold saline immediately and kept at 4 °C. The following methods were performed as follows.

2.10.1. Lipid peroxidation

Lipid peroxidation was estimated by the method of Ohkawa[20]. UV spectrphotometer of model No. SPECORD ® 50 PLUS Analytikjena was used. The levels of lipid peroxides were expressed as nmole of thiobarbituric acid-reacting substances/mg protein using extinction co-efficient of 1.56×10^5 L/mol·cm.

2.10.2. Reduced glutathione level

Reduced glutathione was estimated spectrophotometrically by determination of dithiobis (2-nitrobenzoicacid) reduced by sulfhydryl-groups, as described by Mulder and expressed as nmol/mg protein[21]. Outside diameter was read [within 2-3 min after the addition of 5,5′-dithiobis-(2-nitrobenzoic acid)] at 412 nm in UV Spectrophotometer of model No. SPECORD ® 50 PLUS Analytikjena against a reagent blank. Appropriate standards were run simultaneously.

2.10.3. Catalase activity

Catalase activity was measured based on the ability of the enzyme to break down H_2O_2. Sample with 10 μL was taken in tube containing 3.0 mL of H_2O_2 in phosphate buffer. Time required for 0.05 optical density changes was observed at 240 nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer [0.16 mL H_2O_2 (30% w/v) was diluted to 100 mL of phosphate buffer]. The absorbance was noted at 240 nm after the addition of enzyme; Δt was

noted till outside diameter was 0.45. If Δt was longer than 60 seconds, the procedure was repeated with more concentrated enzyme sample. Reading was taken at every 3 seconds interval. A unit catalase activity is the amount of enzyme that liberates half of the oxygen peroxide from H_2O_2 solution of any concentration in 100 seconds at 25 °C. Catalase activity is expressed as follows[22].

Moles of H_2O_2 consumed/min (Units/mg protein)=2.3/Δt×In (E initial/E final)×1.63×10^{-3}

where E is optical density at 240 nm; 2.3 is factor to convert in to log; Δt refers to time required for a decrease in the absorbance.

2.11. Histopathological study

2.11.1 Tissue preparation for histology

After sacrificing the rats by cervical decapitation, pancreatic tissues were collected, washed in normal saline and fixed by using fixative (picric acid, formaldehyde 40% and glacial acetic acid) for 24 h and dehydrated with alcohol. All tissues were cleaned and embedded by using xylene and paraffin (melting point 55-60 °C). Tissues were stained by double staining process. To differentiate the nucleus and cytoplasm, the basic due haematoxylin and the acid dye eosin were used[23]. Electron micrographs were performed using transmission electron microscope and photographed by photomicrography.

2.12. Statistical analysis

All result was expressed as the mean±SEM using One-way Analysis of Variance (ANOVA) followed by Dunnett's tests. The software used was Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA, USA). The results are analyzed for statistical significance and significance was expressed by *P* value, as mention in the tables. $P<0.01$ was considered as statistically significant.

3. Results

3.1. Effect of treatment on general parameters

Changes in various physical parameters like the body weight, blood glucose level, biochemical estimations were studied in normal controls, STZ induced untreated and treated (with MECB 250 mg/kg b.w., MECB 500 mg/kg b.w. and standard metformin) diabetic rats.

3.2. Effect of MECB on oral glucose tolerance in normal mice

In OGTT test the blood glucose levels of control group reached a peak increased at 30 min and then increased continuously to attain a basal glucose level. Dose dependent blood glucose reduction was observed in animals treated with 250 mg/kg and 500 mg/kg at 60, 90 and 120 min. All the doses showed significant reduction in blood glucose ($^{**}P<0.01$) when compared to control (Table 1).

Table 1

Oral glucose tolerance test. mg/dL.

Animal group	0 min	30 min	60 min	90 min	120 min
Group-I	77.000±0.547	141.400±0.927	152.6±1.122	162.800±0.969	181.600±1.435
Group-II	79.200±1.319	101.600±3.473**	101.2±1.715**	94.400±0.748**	84.200±2.782**
Group-III	80.000±0.836	111.000±1.871**	102.2±2.538**	92.000±0.836**	81.200±0.734**

Values are expressed as mean±SEM (n=5); Statistical significance: *: $P<0.05$; **: $P<0.01$, compared with Group-I.

3.3. Effect of MECB on body weight

The body weight of STZ-induced diabetic rats decreased gradually; on the other hand, the animals of test groups showed signs of improvement in their body weights progressively during 15 d treatment except on the 5th and 10th day. The standard group (Group-V) animals showed improvement in their body weights gradually as compared with normal control group (Group-I) (Table 2). In diabetic control group (Group-II), body weight decreased gradually throughout the 15 d experiment from (182.400±1.470) to (174.400±2.205) g. After administration of MECB (250 mg/kg b.w.) there was a slight increase in body weight from (191.400 ±2.462) to (192.800±2.683) g and after the administration of high dose (500 mg/kg), body weight increased from (182.600± 2.750) to (186.200±2.634) g during the 15 d treatment. Standard treated group (Group-V), showed increase in body weight from (170.600±2.804) g on 0th day to (175.800±4.306) g on 15th day.

Table 2

The deviation of body weight of the animals during the treatment of MECB during 15 days of treatment. mg/dL.

Animal group	0th day	5th day	10th day	15th day
Group-I	190.600±2.804$^{\#\#}$	191.600±2.804$^{\#\#}$	192.600±0.390$^{\#\#}$	195.600±2.205$^{\#\#}$
Group-II	182.400±1.470$^{\#}$	181.200±1.594*	176.600±2.337**	174.400±2.205**
Group-III	191.400±2.462$^{\#\#}$	190.000±2.739$^{\#\#}$	191.600±2.619$^{\#\#}$	192.800±2.683$^{\#\#}$
Group-IV	182.600±2.750$^{\#\#}$	181.200±02.245*	185.000±2.510	186.200±2.634
Group-V	170.600±2.804**	171.000±3.674**	174.000±4.000**	175.800±4.306**

Values are expressed as mean±SEM (n=5); Statistical significance: *: $P<0.05$; **: $P<0.01$, compared with that of normal control Group-I; $^{\#}$: $P<0.05$; $^{\#\#}$: $P<0.01$, compared with standard Group-V.

3.4. Effect of MECB on blood glucose level

In Group-II, blood glucose level of animals increased gradually throughout the 15 days experiment from 242.600 to 270.000 mg/dL. After administration of MECB (250 mg/kg b.w.) there was a decrease in blood glucose level from 242.400 to 135.200 mg/dL in Group-III, but after the administration of high dose (500 mg/kg) blood glucose level decreased from 234.000 to 123.800 mg/dL in Group-IV during the 15 d

treatment. Group-V showed a drastic fall in blood glucose level from (240.000±2.739) mg/dL on 0th day to (113.2±1.200) mg/dL on 15th day (Table 3, Figure 1).

Table 3

The effect of MECB on fasting blood glucose level on STZ-induced diabetic rats. mg/dL.

Treatment	0th day	5th day	10th day	15th day
Group-I	92.530±0.447	102.000±0.547$^{\#\#}$	102.600±1.070$^{\#\#}$	96.400±0.670$^{\#\#}$
Group-II	242.600±1.122**	264.800±1.463$^{**\#\#}$	270.000±2.230$^{**\#\#}$	270.000±0.540$^{**\#\#}$
Group-III	242.400±1.122**	177.400±1.122$^{**\#\#}$	173.800±1.590$^{**\#\#}$	135.200±1.463$^{**\#\#}$
Group-IV	234.000±1.871**	160.800±1.855**	132.000±0.890$^{**\#\#}$	123.800±2.035$^{**\#\#}$
Group-V	240.000±2.739**	164.000±1.871**	132.600±1.120**	113.200±1.200**

Values are mean±SEM (n=5); Statistical significance: *: P<0.05; **: P<0.01, compared with Group-I; $^{\#}$: P<0.05; $^{\#\#}$: P<0.01, compared with Group-V.

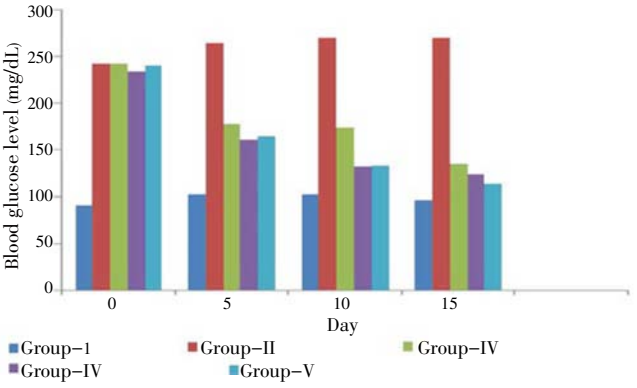

Figure 1. Blood glucose level.

3.5. Effect of MECB on plasma lipid profiles and serum biomarkers

Table 4 illustrates the effects of different doses (250 mg/dl and 500 mg/dl) of MECB on the serum cholesterol, serum triglyceride, HDL and LDL levels in STZ-induced diabetic rats using metformin hydrochloride (10 mg/kg) as standard drug. In the present study, the total cholesterol, triglycerides and LDL cholesterol was increased in diabetic control groups and it was reduced in 15 d treatment with MECB but the HDL cholesterol level was significantly increased. On treatment with MECB there were decreased triglyceride and cholesterol levels compared to those of diabetic control Group-II. The HDL-cholesterol level increased and LDL cholesterol level decreased significantly as compared with diabetic control Group-II.

Table 4

Effects of MECB on plasma lipid profiles (cholesterol, triglycerides, HDL, and LDL) in STZ-induced diabetic rats. mg/dL.

Groups	Cholesterol	Triglyceride	HDL	LDL
Group-I	158.200±0.916$^{\#\#}$	207.600±1.122$^{\#\#}$	41.000±2.300$^{\#\#}$	67.640±3.800$^{\#\#}$
Group-II	228.200±0.916**	300.200±0.916**	26.000±2.200**	137.530±4.200**
Group- III	172.600±1.122$^{**\#\#}$	236.400±1.568$^{**\#\#}$	32.172± 0.368$^{\#}$	91.500±1.200$^{*\#\#}$
Group-IV	205.200±1.463$^{**\#\#}$	250.400±0.748$^{**\#\#}$	24.000±0.316**	122.420±1.993$^{\#\#**}$
Group-V	165.800±0.969$^{**\#\#}$	232.600±1.122$^{**\#\#}$	34.000±1.700$^{\#}$	84.580±3.800$^{*\#\#}$

Values are mean±SEM (n=5); Statistical significance: *: P<0.05; **: P<0.01, compared with Group-I; $^{\#}$: P<0.05; $^{\#\#}$: P<0.01, compared with Group-II.

3.6. Effect of MECB on in vivo antioxidant status

The oxidative stress induced by STZ may lead to imbalance of *in vivo* antioxidant system and that was evaluated by this study. The oxidative stress in diabetic rats was measured by markers since free radical measurement was difficult due to their very short half-life and their low concentration shown in Table 5.

Table 5

Effect of MECB on liver lipid peroxidation, glutathione and catalase enzymes in STZ-induced diabetic rats.

Groups	Lipid peroxidation (nmol/L of MDA/mg of protein)	Glutathione (nmol/Lmg of protein)	Catalase (nmol/L of H_2O_2 decomposed/min/mg)
Group-I	15.410±0.244$^{\#\#}$	50.800±0.800$^{\#\#}$	14.200±0.800$^{\#\#}$
Group-II	35.600±0.400$^{**\#\#}$	23.800±1.020$^{**\#\#}$	5.600±0.400$^{**\#\#}$
Group-III	27.400±0.400	35.200±0.800**	5.800±0.200$^{**\#\#}$
Group-IV	23.800±0.489$^{**\#\#}$	7.600±0.244$^{**\#\#}$	7.800±0.734**
Group-V	20.000±0.632$^{**\#\#}$	43.600±1.470$^{**\#\#}$	8.400±0.244**

Values are mean±SEM (n=5); Statistical significance: *: P<0.05; **: P<0.01, compared with Group-I; $^{\#}$: P<0.05; $^{\#\#}$: P<0.01, compared with Group-V.

Elevated lipid peroxidation was studied in STZ diabetic animals. Groups treated with MECB and metformin significantly reduced the oxidation of lipids in liver.

Total antioxidant status was measured by amount of enzymatic glutathione. Glutathione played the important role in balance the oxidative stress. In diabetic control groups, the decreased glutathione may be due to reduction in glutathione synthesis or degradation of glutathione by oxidation stress in diabetic animal. Diabetic group produced less glutathione activity whereas the standard drug showed reductase activity.

Elevation of biomarker enzymes such as SGOT, SGPT, ALP were observed in diabetic group which indicated hepatic damage. On treatment with MECB the reduced levels of the elevated marker enzymes *i.e.* SGOT, SGPT, ALP had been restored to more or less normal values which indicated recovery of insulin secretion shown in Table 6. Hence MECB may act as an antihyperglycemic agent.

Table 6

Effect of MECB on serum biomarkers in STZ-induced diabetic rats.

Groups	SGOT	SGPT	ALP
Group-I	52.600±1.122$^{\#\#}$	40.800±0.969$^{\#\#}$	104.200±0.374$^{\#\#}$
Group-II	105.00±2.236**	82.400±0.748**	172.600±1.122**
Group-III	74.200±1.356$^{**\#\#}$	62.600±1.122$^{**\#\#}$	135.000±1.095$^{**\#\#}$
Group-IV	55.600±1.122$^{\#\#}$	47.400±0.748$^{**\#\#}$	121.800±1.594$^{**\#\#}$
Group-V	51.800±0.916$^{\#\#}$	46.600±0.672$^{**\#\#}$	120.400±0.748$^{**\#\#}$

Values are mean±SEM (n=5); Statistical significance: *: P<0.05; **: P<0.01, compared with Normal control group I; $^{\#}$: P<0.05; $^{\#\#}$: P<0.01, compared with Group-II.

Catalase is an antioxidant enzyme produced naturally within the body. It helps the body to convert hydrogen peroxide into water and oxygen, thus preventing the formation of carbon dioxide bubbles in the blood. Catalase also uses hydrogen peroxide to break down potentially

harmful toxins in the body, including alcohol, phenol, and formaldehyde. It is a haem containing enzymes. The level of catalase was improved by MECB and metformin. The above *in vivo* antioxidant status is a support to antihyperglycemic effect of methanolic bark extract of *C. bejolghota*.

3.7. Effect of MECB on histopathology

3.7.1. Group I normal control

The slide on normal control (Figure 2) shows presence of cell compactness and there is no cell integrity. The islet boundaries are clear and the profiles of the islet cells are clearly visible. The duct area is prominent without necrosis or fatty degeneration observed. The acinar cells are stained strongly and found to be arranged in lobules with prominent nuclei. The islets cells were found to be embedded in among the acinar cells. The overall architecture was found to be normal and healthy.

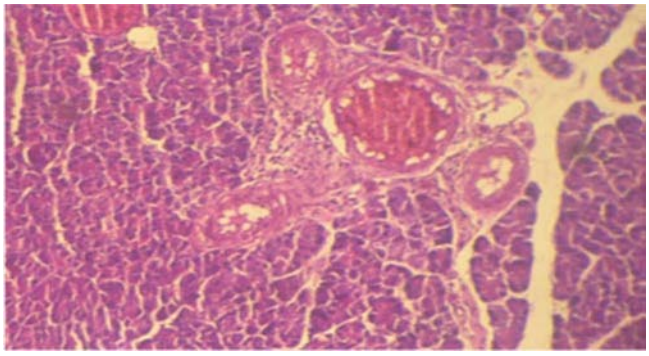

Figure 2. Normal control.

3.7.2. Group II diabetic control

Cell size was found to be shrunken and severe architectural disarray or damage which was observed along with absence of islets cells (Figure 3). The pancreatic cell degradation is prominent in this slide. The cell integrity is total loss. Significant fatty layer degradation has occurred. Normal eco-structure has been lost, and central lobes are destroyed; normal cellular integrity is completely lost. Irregular gap junctions appeared, coagulation occurred due to necrosis. The cell disarrangement and architectural damage seem to be due to the induction of streptozotocin. The photomicrograph also revealed infiltration of lymphocytes indicating severe damage.

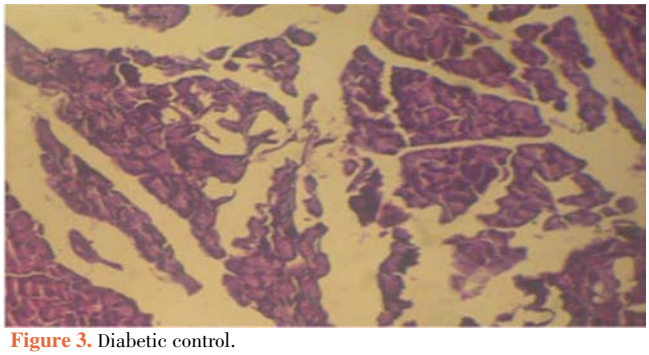

Figure 3. Diabetic control.

3.7.3. Group III

Fatty layers degradation has noticed. The duct area is prominent and ballonic present. The compactness of cells is comparatively better than diabetic control (Figure 4). In most portions cellular integrity is normal with slight fatty degeneration. Disarrangement of architecture was observed without islets cells around with some artifacts of fixation. The remaining acinar cells were observed to be normal as compared positive control group.

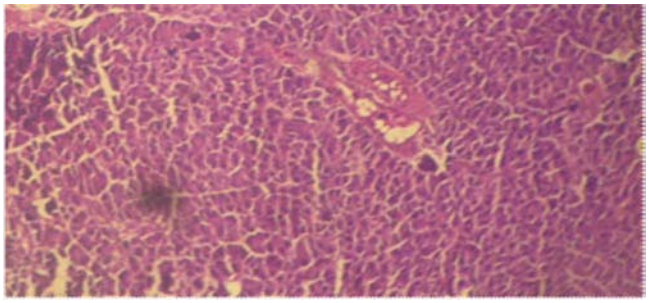

Figure 4. Test control (low dose).

3.7.4. Group IV

The architecture of cells were reverted back to normal with a large proportion of islets cells observed in good health. The acinar cell arrangements were found to be normalized sufficiently as compared to positive control group (Figure 5).

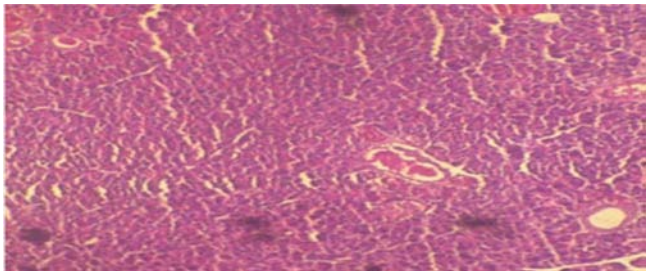

Figure 5. Test control (high dose).

3.7.5. Group V standard drug treated

The islets cells in standard drug treated group were observed as normal in position. But number of islets cells were seems to be less comparative to the normal group. The architecture of acinar cells and size were back to normal upon the standard drug treatment. The overall cellular arrangement were found to be normalized (Figure 6).

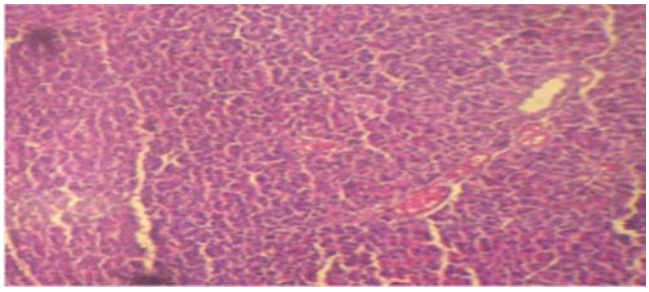

Figure 6. Standard control.

4. Discussion

The study was undertaken to evaluate antihyperglycemic effect of MECB in normal controls, STZ-induced untreated and treated diabetic rats.

Diabetes mellitus is mainly caused by the islet dysfunction and insulin resistance[24]. It is a group of metabolic diseases, and divided into two categories: type 1 and type 2. These two types of diabetes have a distinct pathogenesis, but hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are their most common features. More than 90% of diabetic patients are type 2 diabetes suffering from severe insulin resistance[25]. From OGTT it could be concluded that dose 500 mg/kg showed maximum improvement in glucose tolerance. STZ significantly induced hyperglycaemia. Oral administration of MECB for 15 d caused a significant decrease in blood glucose levels. The possible mechanism by which MECB mediated its antidiabetic effect could suppress hepatic gluconeogenesis and glucose output from liver. The hypoglycaemic of MECB was compared with metformin, a standard hypoglycaemic drug. From the present study it may be suggested that the mechanism of action of MECB may be similar to metformin action. The patient with diabetes or diabetic animal model exhibited the attenuation of antioxidant capacity and/or immune function, and then disturbed lipid metabolism function[26]. The results in this study indicated that there was apparent reduction of serum levels of triglyceride, total cholesterol, LDL in treated groups whereas the HDL level increased. Elevation of biomarker enzymes such as SGOT, SGPT, ALP were observed in diabetic group which indicated hepatic damage. On treatment with MECB the reduced levels of the elevated marker enzymes *i.e.* SGOT, SGPT, ALP had been restored to more or less normal values which indicated recovery of insulin secretion. Hence MECB may act as an antihyperglycemic agent. It is widely believed that catalase and glutathione are the important enzymes of scavenging oxygen free radicals in organism, and protect the pancreatic tissue against oxidative stress injury. In addition, the content of malondialdehyde reflects the degree of the organic lipid peroxides and cell damage. Our data found that the MECB could improve catalase and gluthione activities in pancreatic tissue of diabetic mice and lower the malondialdehyde content, noting that MECB treatment had beneficial effects on diabetes by directly scavenging free radical. The pathological examination of the pancreatic section using helium-staining exhibited that the islet in the normal control group displayed the complete structure and uniform arrangement with numerous pancreatic beta cells. Normal compactness was found. In contrast, pathology of islet in diabetic control group showed damaged with loosening or deforming, and the reduction of pancreatic beta cells. Severe architectural disarray or damage was observed. The structure of islet was partially improved and then number of pancreatic beta cells was augmented in low dose treated group (250 mg/kg b.w.). The islet structure was clearer restoring and the quantity of pancreatic beta cells was gradually increased in high dose treated group (500 mg/kg b.w.). The islets cells of group treated standard drug were observed as normal in position. But number of islets cells seems to be less comparative to the normal group. The architecture of acinar cells and size were back to normal upon the standard drug treatment.

Thus the significant antihyperglycemic activity of *C. bejolghata* could be due to the presence of various phytoconstituents detected in the phytochemical screening. From the study, it can be concluded that MECB has beneficial effect on blood glucose level. It has potential to impart therapeutic effect on diabetes. Further studies are necessary to elucidate in detail the mechanism of action of this medicinal plant at cellular and molecular level. It is also necessary to isolate responsible active constituents to establish its mechanism of action. It is clear from the studies that the plant possesses promising antidiabetic activities, so, furthermore studies are required for establishing this important plant as a potent source for medicinal use.

The present data suggested that MECB has an antihyperglycaemic effect in type-2 diabetic rats. Oral administration of MECB to the diabetic rats not only significantly lowered the blood glucose levels, but also caused improvement in lipid profile and other biochemical parameters. Meanwhile, the glucose tolerance and *in vivo* antioxidant status were also improved.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are thankful to Department of Pharmaceutical Sciences, Dibrugarh University, India for providing the research facilities for this work and All India Council of Technical Education, Research Promotion Scheme 2011,

Grant No.: 8023/RIB/RPS-10/(NER)/2011-12 for providing grant. The authors take the opportunity to acknowledge the support of Dr. Giriraj Kusre, Associate Professor, Dept. of Anatomy, Assam Medical College for his immense help and support in conducting a part of the research work in his special facility of Diagnostic Genetic Lab, Dept of Anatomy, Assam Medical College.

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